

Fig. 1

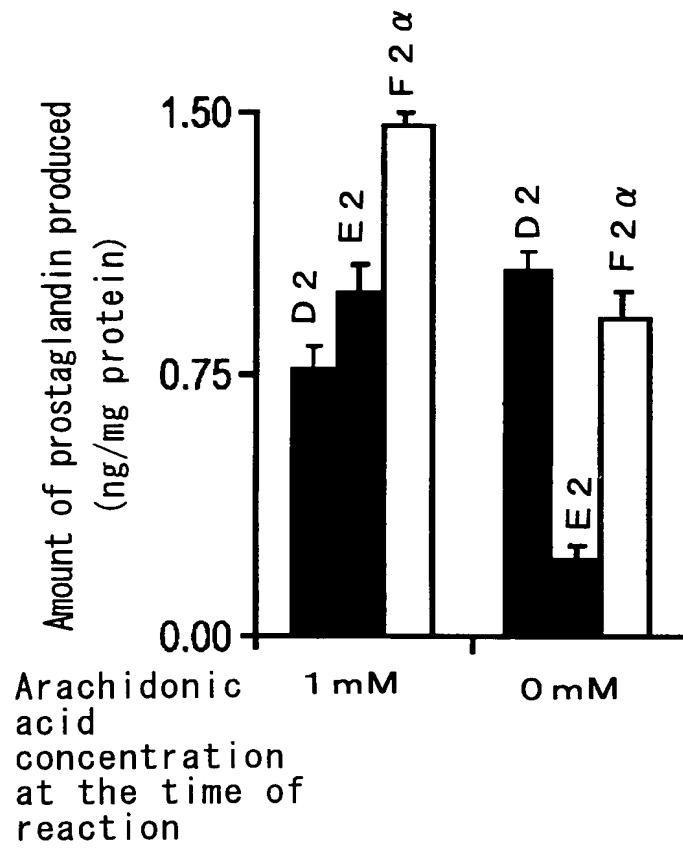
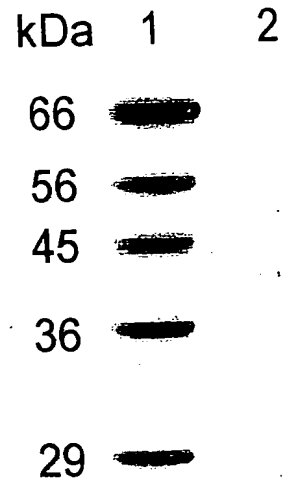




Fig. 3



1. Molecular-weight marker protein
2. Purified enzyme

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Fig. 4

Purification step	Total protein (mg)	Enzymatic activity (nmol/min)	Specific activity (nmol/min/mg protein)	Purification ratio
Soluble fraction	171.0	154	0.9	1.0
20-80% saturation ammonium sulfate fraction	127.0	150	1.2	1.3
Superdex 200	113.0	150	1.2	1.3
Ultrafiltration chromatogram	8.0	170	25.0	28.0
Hydrophobic chromatogram				
DEAE ion exchange chromatogram	2.8	180	64.0	71.0
Superdex 200				
Ultrafiltration chromatogram	0.3	210	700.0	778.0
2nd time				

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Fig. 5

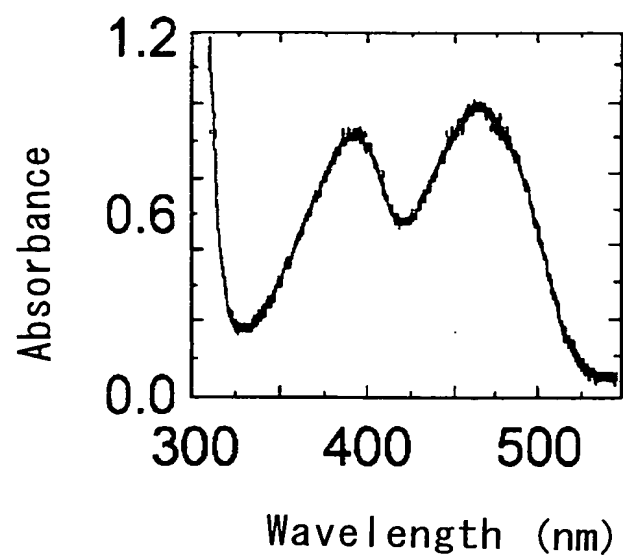
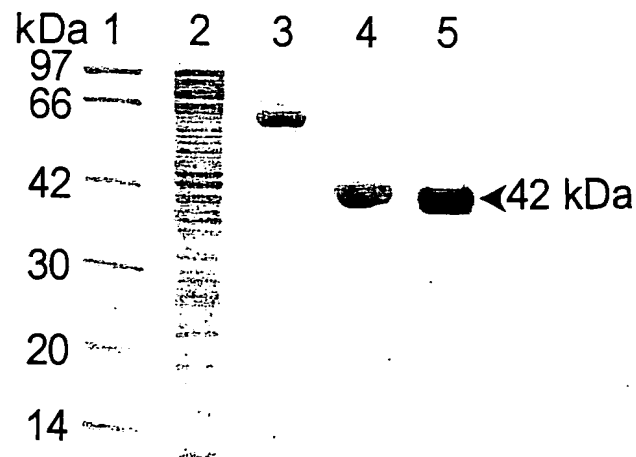


Fig. 6



1. Molecular weight marker protein
2. E. coli crude extract after transformation
3. Crude extract of E. coli expressing the recombinant TcOYE
4. Recombinant TcOYE collected by thrombin treatment
5. Purified standard of the recombinant TcOYE

Fig. 7

## Substrate specificity of reduction by the recombinant TcOYE

Substrate	Cofactor (10 $\mu$ M)	K <sub>m</sub> ( $\mu$ M)	V <sub>max</sub> /specific activity (nmol/min/mg)
9,11-endoperoxide PGH <sub>2</sub>	NADH	—	5 5 4
	NADPH	5. 0	7 6 6
Hydrogen peroxide BHP <sup>a</sup>	NADPH	2. 3	9 9
	NADPH	n. d.	2 8 2
Menadione	NADH	—	4 9 9
	NADPH	0. 8 2	7 0 0
$\beta$ -lapachone	NADH	0. 1 7	6 5 0
	NADPH	—	4 3 3
4-nitroquinolin-N-oxide	NADH	—	7 5 9
	NADPH	9. 5	1 1 1 0
Nifurtimox	NADH	—	2 9 0
	NADPH	1 9. 0	3 5 3
Phenazine methosulfate <sup>b</sup>	NADPH	1 0. 4	2 3 5
Mevinolin <sup>c</sup>	NADH	n. d.	5 5 5
12-oxo-phytodienoic acid <sup>d</sup>	NADPH	n. d.	1 5 2
9-oxo ODE <sup>e</sup>	NADPH	n. d.	5 4
Econazole <sup>f</sup>	NADH	n. d.	4 3
Benznidazole	—	n. d.	N. D.
Miconazole <sup>g</sup>	—	n. d.	N. D.
Ketoconazole <sup>h</sup>	—	n. d.	N. D.
Crystal violet <sup>i</sup>	—	n. d.	N. D.
BHT <sup>i</sup>	—	n. d.	N. D.
BHA <sup>k</sup>	—	n. d.	N. D.

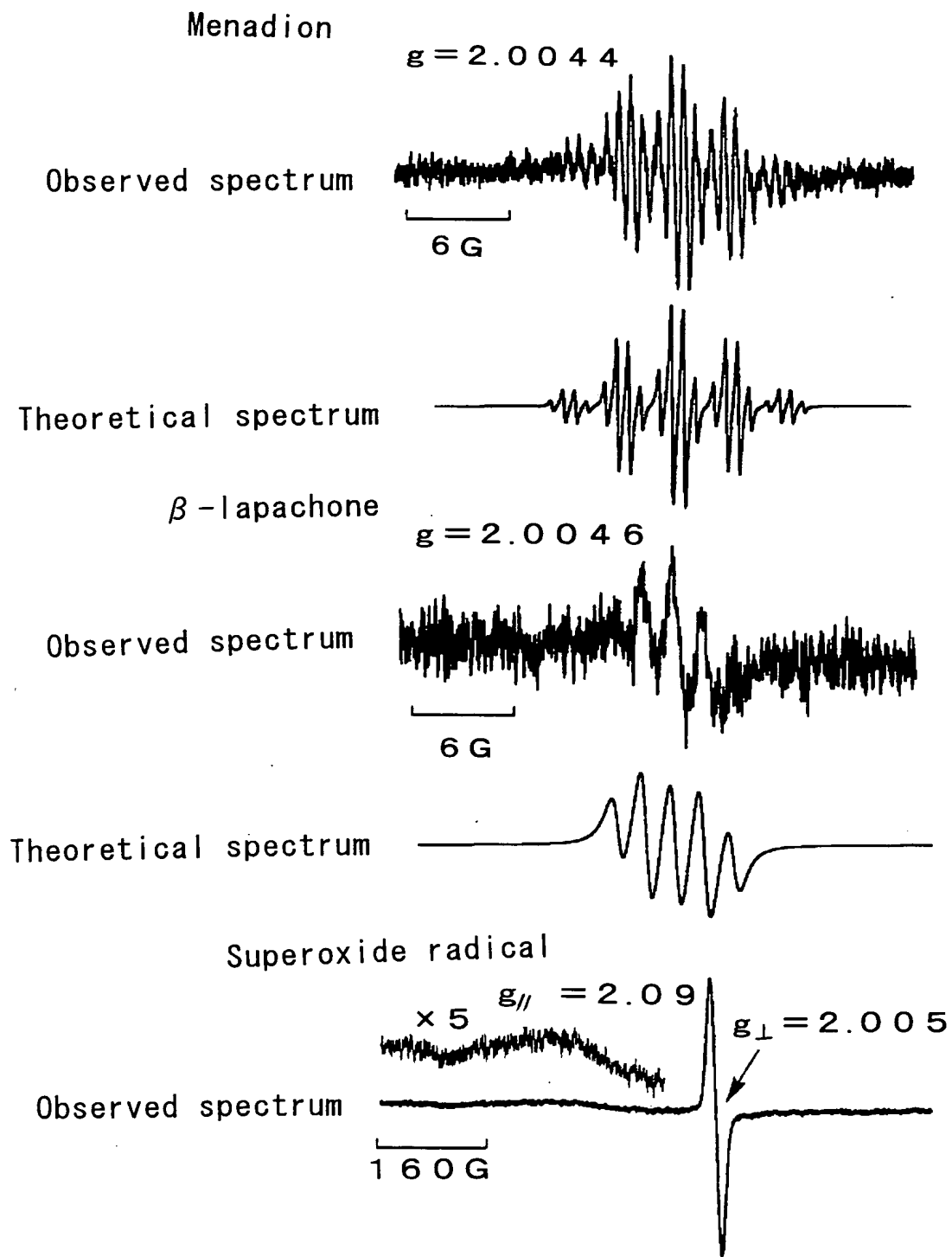
a: t-butyl hydroperoxide, b: 5-methyl-phenaziummethylsulfate), c: 2- $\beta$ , 6 $\alpha$ -dimethyl-8 $\alpha$ -(2-methyl-1-oxo-butoxy)-mevinic acid lactone), d: 4-oxo-5 $\beta$ -(2Z-pentenyl)-2-cyclopentene-1 $\beta$ -octanoic acid), e: 9-oxo-10E, 12Z-octadecadienoic acid, f: 1-[2-([4-chlorophenyl] methoxy)-2-(2,4-dichlorophenyl)ethyl-1H-imidazole], g: 1-[2,4-dichloro  $\beta$ -([2,4-dichlorobenzyl]-oxo)phenethyl] imidazole, h: cis-1-acetyl-4[4-[2-(2,4-dichlorophenyl)-2-(1H-imidazol-1-yl-methyl)-1,3-dioxolane-4-yl-methoxy]phenyl]piperazine], i: N-[4-[bis[4-(dimethylamino)-phenyl]methylene]-2,5-cyclohexadiene-1-yl-iden-N-methyl-methane aluminum chloride, j: (2,6di-tert-butyl-para-crezol), k: [2(3)-tert butyl-4-hydroxyanisole

N. D. : not detected

n. d. : not measured

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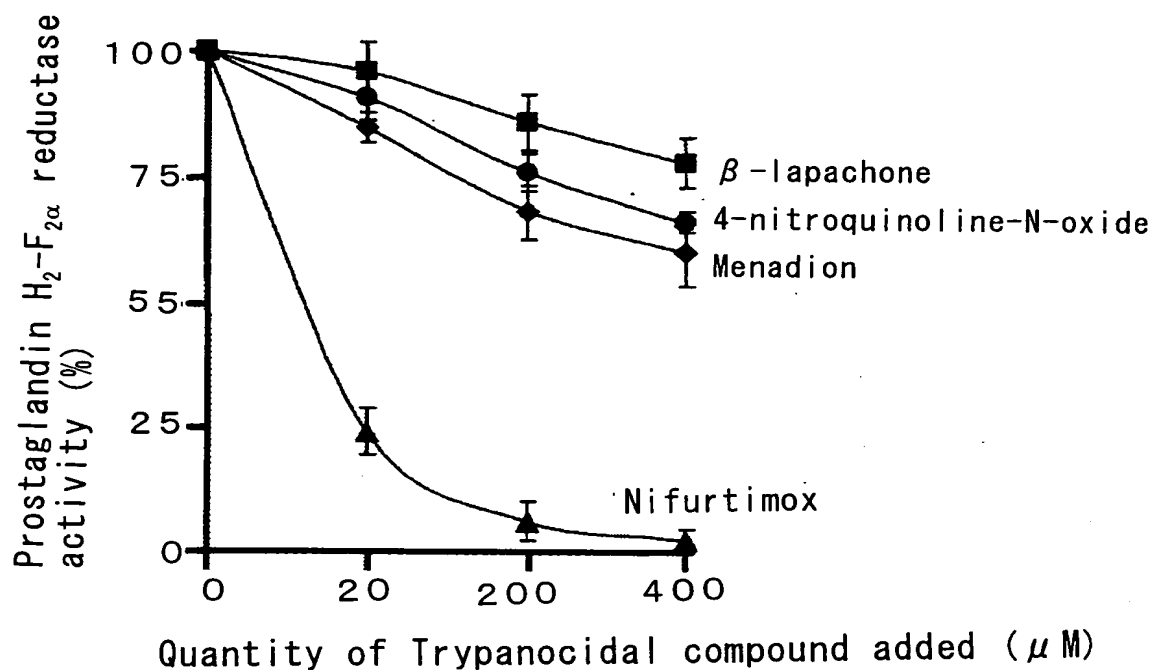
Fig. 8





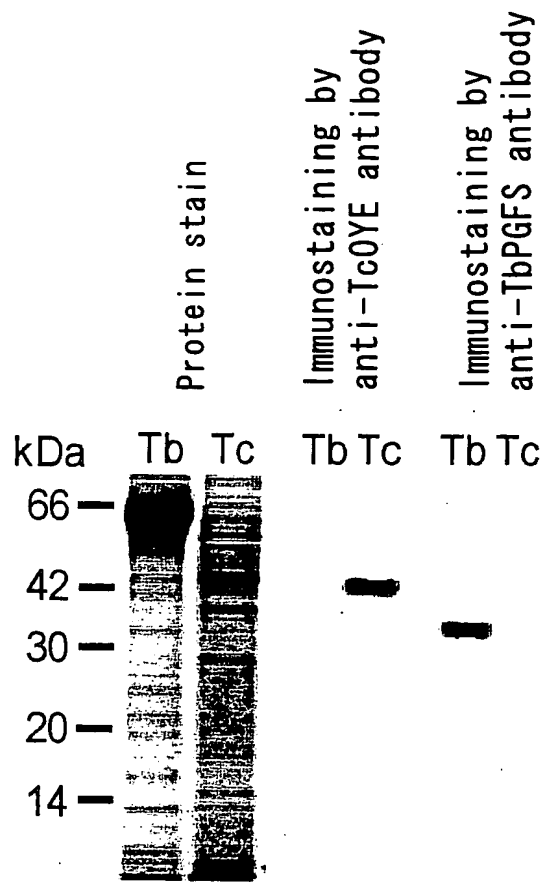
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Fig. 9



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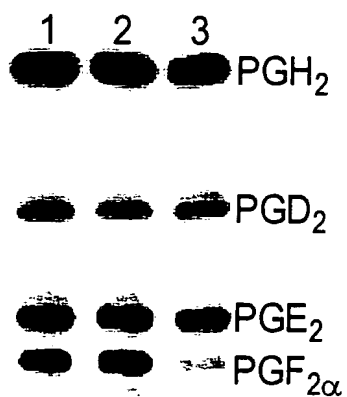
Fig. 10



Tb: Crude extract of *Trypanosoma brucei*

Tc: Crude extract of *Trypanosoma cruzi*

Fig. 11



1. Trypanosoma cruzi crude extract after reaction with control IgG
2. Trypanosoma cruzi crude extract after reaction with anti-TbPGFS antibody
3. Trypanosoma cruzi crude extract after reaction with anti-TcOYE antibody

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Fig. 12

Immunoprecipitation by the anti-TcOYE antibody of the enzymatic activity to reduce menadion,  $\beta$ -lapachone, nifurtimox, 4-nitroquinoline-N-oxide in the crude extract of *Trypanosoma cruzi*

Sample	Persistent enzymatic activity (%)			4-nitroquinoline-N-oxide
	Menadion	$\beta$ -lapachone	Nifurtimox	
Trypanosoma cruzi extract after reaction with anti-TcOYE antibody	<sup>a</sup> N. D.	10( $\pm$ 2)	N. D.	N. D.
Trypanosoma cruzi extract after reaction with anti-TbPGFS antibody	98( $\pm$ 8)	103( $\pm$ 3)	100( $\pm$ 5)	100( $\pm$ 10)
Trypanosoma cruzi extract after reaction with control bovine IgG	100( $\pm$ 4)	100( $\pm$ 6)	100( $\pm$ 10)	100( $\pm$ 6)